Plant Pathology Journal 9 (1): 14-22, 2010
ISSN 1812-5387

Analysis and RT-PCR Identification of Viral Sequences in Peanut (*Arachis hypogaea* L.) Expressed Sequence Tags from Different Peanut Tissues

[1]P.M. Dang, [2]B.T. Scully, [1]M.C. Lamb and [2]B.Z. Guo
[1]US Department of Agriculture, Agricultural Research Service,
National Peanut Research Laboratory, Dawson, GA 39842, USA
[2]US Department of Agriculture, Agricultural Research Service,
Crop Protection and Management Research Unit, Tifton, GA 31793, USA

Abstract: In the Southeastern US, peanut plants are often naturally infected with different viruses resulting in economic losses. Limited information is available concerning the identification and characterization of various viruses in peanut. A collaborative effort has resulted in the production of 44,064 expressed sequence tags (ESTs) from developing seeds at three reproductive stages (R5, R6 and R7) of two peanut genotypes, Tifrunner and GT-C20 and from field collected leaf tissues using the same two genotypes. EST sequences were analyzed, assembled and putative functions were determined based on the comparison of these unique peanut sequences with the National Center of Biotechnology Information (NCBI) non-redundant (nr) database using BLASTX program. Sequences matched viral or viral associated proteins were selected for further study. We analyzed the extent of viral sequences in these peanut ESTs and detected the sequences of *Peanut mottle virus* (PMV), *Peanut stripe virus* (PStV) and *Tomato spotted wilt virus* (TSWV). A total of 942 sequences were identified that matched viral sequences in GenBank to PMV (606), PStV (330) and TSWV (6). We also identified peanut plant sequences with homology to pea (*Pisum sativum*) plant-specific Potyvirus VPg-interacting protein (*PVIP*), *Arabidopsis thaliana* tobamovirus multiplication protein 3 (*TOM3*) and a tobacco TMV helicase domain-binding protein. These host proteins are essential for viral multiplication and movement from cell to cell in host plants. Further study, we examined and identified the presence of these viral and plant sequences in peanut leaves, seeds and roots using RT-PCR. The potential use of these putative peanut virus host responsive proteins may lead to the development of potentially new strategies to control these virus diseases by silencing or disrupting these genes to prevent virus infection and multiplication in host plants.

Key words: Peanut, ESTs, viral sequences and detection, RT-PCR

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the major economically-important legumes. It is grown widely in the Southern United States, India, China and many countries in Africa and South America, where peanut is able to grow in semi-arid environments with minimal use of chemical fertilizers. Globally, peanut is a major source of protein (25-28%) and vegetable oil (43-55%) for human nutrition. About two thirds of the world's production is crushed for oil and the remaining one third is consumed as food (Bunting *et al*., 1985). High production costs due to chemical control of diseases in the US and food safety concerns from aflatoxin contamination present challenges for successful peanut production. Viral-based diseases are widespread causing significant reductions in yield. For example, a field survey for peanut viruses in Georgia (Kuhn *et al*., 1984) found *Peanut mottle virus* (PMV), *Peanut stripe virus* (PStV) and *Peanut stunt virus* (PSV), but did not detect *Tomato spotted wilt virus* (TSWV). These viruses reduce yield and are sometimes a major limiting factor in peanut production.

Tospoviruses, such as TSWV, cause major diseases of peanut worldwide and have become increasingly important in the production of peanut in the Southeastern United States. TSWV has been one of the most serious and complex disease problems in peanut since 1985 in the Southern US (Culbreath *et al*., 1997). In 1997, peanut losses from TSWV were estimated at $40 million for Georgia alone. Another widespread virus on peanut is PMV, a potyvirus. Its transmission through infected seed is assumed to be the reason for its worldwide distribution

Corresponding Author: Baozhu Guo, US Department of Agriculture, Agricultural Research Service,
Crop Protection and Management Research Unit, Tifton, GA 31793, USA
Tel: 229-387-2334 Fax: 229-387-2321

(Gillaspie *et al*., 2000). The PMV was reported to infect peanut plants in Georgia in 1961 and the virus was found to be seed-transmitted at a rate of 2% which was calculated to reduce yield by about 25% in experimental plots (Kuhn, 1965). PStV, another potyvirus (Pappu *et al*., 1998), was found in the United States in field-grown peanut in 1982 (Demski and Lovell, 1985). Seed transmission was over 5% in some plots resulting in reductions in yield in Georgia of about 7% in experimental plots (Lynch *et al*., 1988).

Current technology in molecular biology have allowed researchers to capture snapshots of what is important to plant cells under a certain condition due to adverse environmental challenge or at a particular stage of development. Expressed Sequence Tags (ESTs) represent messages that are communicated by the plant cells and can readily be captured by constructing a cDNA library. This strategy has been employed in peanut to understand plant responses to leaf spot disease caused by *Cercosporidium personatum* (Luo *et al*., 2005b) and to *Aspergillus parasiticus* infection and drought stress (Luo *et al*., 2005c). Because of the similarity between plant viral RNA and poly-adenylated plant mRNA, viral RNA molecules containing poly-(adenines) can be incorporated into cDNA libraries and subsequently detected by DNA sequencing (Thompson *et al*., 2004; Valles *et al*., 2004, 2008). The search and analysis of these viral sequences can lead to new breakthrough such as the discovery of a picorna-like virus from the red imported fire ant, *Solenopsis invicta* (Valles *et al*., 2004). Data mining of existing ESTs can identify the presence of viruses, such as the presence of *Bean pod mottle virus*, *Soybean mosaic virus* and *Cowpea chlorotic mottle virus* in various soybean tissues (Stromvik *et al*., 2006).

Recent efforts have led to the sequencing of 10 cDNA libraries from different tissues and different peanut genotypes (Luo *et al*., 2005a; Guo *et al*., 2008, 2009). Up to date, a total of 44,064 ESTs from 10 peanut cDNA libraries have been sequenced. With the availability of a large number of peanut ESTs, the goal of this study is to analyze and examine the unique set of ESTs for the presence of viral sequences and to identify and confirm the presence of viral and other plant sequences using RT-PCR.

MATERIALS AND METHODS

Library information and EST analysis: A total of 44,064 cDNA clones have been sequenced from 10 peanut cDNA libraries (Luo *et al*., 2005a; Guo *et al*., 2008, 2009). There were total six cDNA libraries constructed from Tifrunner (runner type) and GT-C20 (Spanish type) developing seeds at the R5 (beginning seed), R6 (full seed) and R7 (beginning maturity) reproductive stages (Boote, 1982), respectively. Additional libraries were created from Tifrunner leaves, GT-C20 leaves and A13 immature pods (Luo *et al*., 2005a). Tifrunner (previous designated as C34-24) is resistant to *Tomato spotted wilt virus* (TSWV) (Holbrook and Culbreath, 2007) while GT-C20 is susceptible. A13 is a breeding line and has drought tolerance with reduced aflatoxin contamination. Total RNA isolation, library construction and DNA sequencing were previously reported (Luo *et al*., 2005a; Guo *et al*., 2008, 2009). The EST processing, clustering and BLAST analysis for all sequences were performed in 2008. Any contiguous sequences (contigs) and singlets that matched virus or virus-related proteins were selected for further analysis. The final contigs were manually inspected using trace files from raw sequencing data and the consensus sequences were aligned with the complete viral genome reference sequences, using the program BLAST2seq (Altschul *et al*., 1997).

RNA extraction: Tifrunner and GT-C20 were planted in the field at Tifton, GA with standard cultural practices. Plants were subjected to the natural environmental conditions. Plants were harvested for tissue samples of leaves, pods and main root at 100 days after planting. Tissues were stored at -80°C until processing. Approximately 1 g of peanut tissues (leaf, root and seed) was ground separately using mortar and pestle in liquid N_2. For seed tissue, one or two pods were split-open to obtain 1 g of tissue and seed coat was removed prior to RNA extraction. For leaf tissue, multiple leaves from each plant were pooled to obtain 1 g of tissues. For root tissue, slices of the main tap root were selected. Leaf or root total RNA was extracted (Luo *et al*., 2005a) and treated with RNase-Free DNase I (Qiagen, Valencia, CA). RNA concentration was measured by NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE) and quality was determined by RNA gel electrophoresis.

Primer design and RT-PCR: Specific primers were designed based on consensus sequences from the EST contigs and singlet and the viral sequences using Primer3 software program. For the synthesis of first strand cDNA, total RNA (1 μg) was denatured at 95°C for 5 min in the presence of 1 μL (50 μM) of oligo dT primer with a total volume of 12.5 μL and was immediately placed on ice for 2 min. For TSWV reactions, TSWV 722 and 723 primers (50 μM each) (Adkins and Rosskopf, 2002) were used in the place of oligo dT primer. An aliquot of 17.5 μL of RT reaction mixture (6 μL 5X MMLV RT buffer, 8 μL 2 mM dNTP mix, 2 μL 100 mM DTT, 1 μL MMLV-RT [200 u μL^{-1}], 0.5 μL of RNAsin [40 μL^{-1}]) was added.

The RT reactions were performed at 45°C for 55 min, followed by 5 min at 95°C, 5 min at 22°C and cooled to 4°C. One microliter of RT reaction was used as template for the PCR reaction. An aliquot of 19 μL of PCR reaction mixture (7 μL sterile water, 10 μL Green GOTAQ mix [Promega, Madison, WI], 1 μL forward [10 μM] and 1 μL reverse [10 μM] primers) were added. The PCR profile consisted of a denaturing step at 95°C for 2 min, followed by 30 cycles of 95°C for 45 sec, 55°C for 45 sec, 72°C for 1 min, followed by a 72°C extension for 10 min and a final hold step at 4°C. The PCR products were separated on a 1.2% TAE agarose gel. Gel image was captured on Gel Logic 200 Imaging System (Kodak, Rochester, NY).

RESULTS

From the 44,064 cDNA clones sequenced, 36,616 high quality sequences were obtained after quality and vector trimming. The total peanut EST sequences were clustered into a 10,096 unigene set (contigs and singlets) and searched against the NCBI database. A total of 942 sequences were identified that matched viral and viral plant responsive transcripts. These sequences exhibited significant identity with PMV, PStV and TSWV in GenBank (Table 1).

The largest cluster (contig 0008) contained 605 sequences with 3.9 kb in length. The consensus sequence matched to PMV strain M with an E value of 0 when searched against NCBI sequence database (BLASTn, 96% identity) and protein database (BLASTx, 98% identity). Out of the 605 sequences, 586 sequences were derived from two leaf cDNA libraries which represent 4.06% of all the leaf ESTs (14,432) and the remaining 19 sequences were derived from seed cDNA libraries, which represent 0.085% of all seed ESTs (22,184). The PMV complete genome sequence (AF023848) (Flasinski and Cassidy, 1998) was downloaded from NCBI and compared to the viral sequences. The consensus sequence aligned with the 3'-end of the viral genome which includes Nuclear Inclusion a (NIa), Nuclear Inclusion b (NIb) and Coat Protein (CP) (Fig. 1) and one sequence (C20L-047_H01) that matched to PMV was not in this consensus sequence. The trace file of this singlet was examined for verification of sequence quality. When the complete PMV genome sequence was used as a reference, the singlet was determined to be 150 bp in the 5' direction of contig 0008 and extended the consensus sequence by another 680 bp (Fig 1a).

The second major cluster (contig 0014) contained 330 sequences with 2.7 kb in length. The consensus sequence matched to PStV with an E value of 0 (BLASTn, 99% identity; BLASTx, 99% identity) (Gunasinghe *et al*., 1994). Out of the 330 sequences, 326 were found in the leaf cDNA libraries which represented 2.26% of all leaf ESTs and the remaining 4 sequences were derived from seed cDNA libraries. The complete PStV genome sequence (U05771) was downloaded from NCBI and compared with the putative viral sequences. The consensus sequence aligned with the 3' end of the PStV genome which included a part of NIb and a complete CP (Fig. 1). This sequence contained an additional 250 bp and a poly(A^+) tail that was not included in the downloaded sequence at the 3' end. One sequence (C20L-007_E03) matched to PStV but was not in this consensus sequence. The trace file for this singlet was also analyzed for confirmation of sequence quality. The singlet was determined to be part of the P3 protein located upstream of contig 0014 consensus sequence (Fig. 1b).

The third cluster (contig 0778) is comprised of 5 sequences with 1.0 kb in length. This consensus sequence matched TSWV nucleocapsid (N) protein (AAC33560) with an E value of e^{-137}. The N protein and

Table 1: Frequencies of *Peanut mottle virus* (PMV), *Peanut stripe virus* (PStV), *Tomato spotted wilt virus* (TSWV) and peanut plant-sequences with homologues to pea (*Pisum sativum*) plant-specific *Potyvirus* VPg-interacting protein gene (PVIP), to *Arabidopsis thaliana Tobamovirus* multiplication protein 3 (TOM3) and to *Arabidopsis* TMV helicase domain-binding proteins in peanut cDNA libraries

	Putative function[1]	C20L[2]	TFL	C20R5	C20R6	C20R7	TFR5	TFR6	TFR7	ISBL
Contig0008[3]	PMV	309	277	2	1	3	3	0	3	7
Contig0014	PStV	181	144	2	0	0	0	2	0	0
Contig0778	TSWV	0	0	1	1	0	2	0	1	0
Contig2001	PVIP	2	0	0	0	1	1	0	0	0
Contig1534	TOM3	0	0	1	0	0	1	0	0	1
C20L-006-B05	TMV	1								
C20L-047_H01	PMV	1								
C20R7-012-F06	TSWV					1				
ISBL006_C04	TOM3									1
C20L-007_E03	PStV	1								
Total ESTs per library		8,328	6,104	4,678	1,977	2,017	6,150	4,230	2,046	1,086

[x]The consensus sequence matched to sequences in GenBank when searched against NCBI sequence database (Blastn) and protein database (Blastx). [y]cDNA library: peanut genotypes GT-C20 (C20), susceptible to TSWV and Tifrunner (TF), resistant to TSWV; L (leaf tissues) and developing seeds at three reproduction stages (R5, R6 and R7); ISBL (immature seeds of A13); *Peanut mottle virus* (PMV), *Peanut stripe virus* (PStV), *Tomato spotted wilt virus* (TSWV); *Tobacco mosaic virus* (TMV); *Potyvirus* VPg-interacting protein (PVIP); Tobamovirus multiplication protein 3 (TOM3). [z]Contig or singlet matching viral sequences or plant responding sequences in GenBank

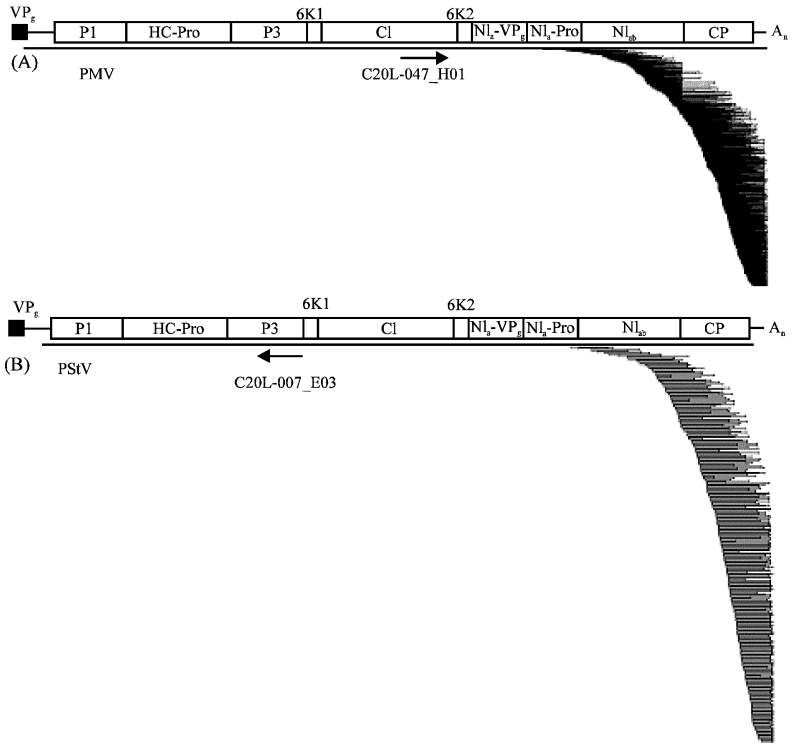

Fig. 1: A schematic organization of the monopartite potyvirus genome, the functional products and alignment of the putative viral sequences in the ESTs. A: the PMV complete genome sequence (AF023848) and the putative viral sequences in the ESTs aligned with the 3'-end of the viral genome; B: the complete PStV genome sequence (U05771) and the putative viral sequences aligned with the 3' end of the PStV genome. Rectangle boxes represent the open reading frame. Vertical lines represent putative polyprotein cleavage sites. HC-Pro, helper component (HC) for vector transmission; P1 and P3, proteinases, Pro, proteinase; CI, cylindrical inclusion protein; NIa, small nuclear inclusion protein; NIb, large nuclear inclusion protein with polymerase (pol) motif; VPg, virus protein genome-linked; CP, coat protein. PMV and PStV are members of the genus *Potyvirus*

the nonstructural (NSs) protein make up the small (S) segment (~3 kb) of the TSWV genome and are encoded in ambisense orientation (Tsompana *et al.*, 2005). The NSs protein is involved in RNA silencing suppressor activity (Takeda *et al.*, 2002) and the N protein is involved in the encapsidation of viral RNA within the viral envelope (Richmond *et al.*, 1998). One singlet (C20R7-012-F06) did not cluster with contig 0778 but matched to G1-G2 precursor glycoprotein (E value of 0) which is part of TSWV segment M. The middle (M) segment (~4.8 kb) of TSWV RNA genome encodes for the NSm protein which is involved in cell-to-cell movement (Kormelink *et al.*, 1994) and the G1 and G2 glycoproteins which play a role in maturation and/or assembly of virions (Bandla *et al.*, 1998).

In addition to viral sequences identified in the collected peanut ESTs, we found transcripts that may be involved in plant response to viral infection and replication. Another cluster (contig 2001) is comprised of 4 sequences with 1.8 kb in length. This consensus sequence matched *Potyvirus* VPg-interacting protein (PVIP) from *Pisum sativum* (AAP22955) with an E value of 0. PVIP is a plant-specific protein with similar proteins found in species such as *Pisum sativum* (pea), *Arabidopsis thaliana* and *Nicotiana benthaminana* (Dunoyer *et al.*, 2004). In *Arabidopsis*, it has been shown that PVIP functions as an ancillary factor that helps potyvirus movement in plants (Dunoyer *et al.*, 2004).

The last cluster (contig 1534) is composed of 3 sequences with 0.85 kb in length and a singlet ISBL006_C04 which is 570 bp in length. These sequences matched to Tobamovirus multiplication protein 3 (TOM3) (NP_027422) with E values of e^{-103} and e^{-82}, respectively. In *Arabidopsis*, mutations in TOM3 genes can significantly suppress tobamovirus coat protein (CP) accumulation (Yamanaka *et al.*, 2002). Contig 1534 and singlet ISBL006_C04 can be clustered together, however, detailed analysis revealed that the number of single nucleotide

polymorphism is relatively high (70/514 bp or 13.6%) and the number of amino acid difference is 26/170 or 15.2%. It is possible that contig 1534 consensus sequence and singlet ISBL006_C04 represent two different forms of Tobamovirus multiplication proteins in peanut. One singlet (C20L-006-B05) sequence matched to TMV helicase domain-binding protein (E value e^{-68}) from tobacco (AF426837.1). This protein belongs to a family of ATPases associated with various activities (AAA) and the silencing of this gene in *Nicotiana benthamiana* caused a twofold reduction of TMV accumulation when challenged with TMV (Abbink *et al*., 2002).

In order to confirm the presence of these viral sequences and plant-responding transcripts, we conducted RT-PCR analysis in different peanut tissues (leaf, seed and root) of Tifrunner and GT-C20 for detection of viral RNAs and host responsive transcripts. Specific primers were designed (Table 2) for viral sequences of PMV and PStV and TSWV specific primers were synthesized according to Adkins and Rosskopf (2002). Primers for plant viral responsive transcripts were also designed for *PVIP*, *TOMs* and TMV helicase domain-binding protein. PMV and PStV transcripts were detected in all replicated samples of seeds, roots and leaves (Fig. 2) for both Tifrunner and GT-C20.

Peanut host viral-responsive transcripts were also detected in all tissue samples, for putative peanut potyvirus VPg interacting protein (ahVPgB), putative peanut Tobamovirus multiplication (ahTOM) protein and putative peanut TMV helicase domain binding protein (ahTMV-Hel BP) (Fig. 2).

Table 2: List of specific primers for RT-PCR to detect viral or plant responsive transcripts

Putative function	Primers	Amplified size (bp)
Peanut mottle virus (PMV)	Forward 5'-GCGGACAGGTTATCTTGCAT	
	Reverse 5'-TGATGTACGCGACGTGATTT	636
Peanut stripe virus (PStV)	Forward 5'-GAACGGCTTTATGGTGTGGT	
	Reverse 5'-GAACCCATGCCAAGAAGTGT	462
Potyvirus VPg interacting protein	Forward 5'-TCAGAAGGTGGAAGGCTGAT	
	Reverse 5'-GCCTTGGGAGGAACACTGTA	536
Tobamovirus multiplication protein	Forward 5'-ACCGTAGCTCTGGTGCAACT	
	Reverse 5'-GACATCAAGGTCGGCATTTT	600
TMV Helicase Domain Binding Protein	Forward 5'-TCCAGACAGCCATCTGTGAT	
	Reverse 5'-TCATTGCAGCCTCTTCACAC	382
Tomato spotted wilt virus (TSWV)	Forward 5'-CACAAGGCAAAGACCTTGAG	
	Reverse 5'-GCTGGAGCTAAGTATAGC	620

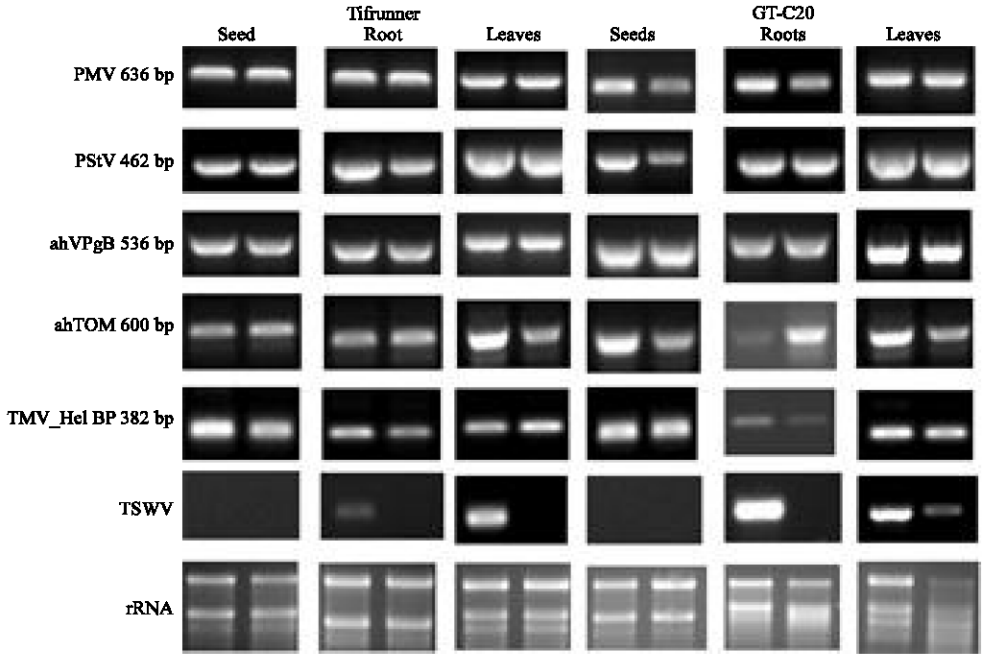

Fig. 2: RT-PCR identification and confirmation of the presence of PMV, PStV and TSWV, along with putative peanut viral-responsive transcripts *ahVPgB*, *ahTOM* and *TMV_Hel BP* in peanut seeds, roots and leaves of peanut cultivar and breeding line, Tifrunner and GT-C20

DISCUSSION

Our initial goals were to isolate transcripts that are differentially expressed in response to drought and *Aspergillus* infection from developing peanut seeds and transcripts that were differentially expressed in the presence of TSWV and leaf spot disease from leaf tissues. In the process of analyzing the collection of EST sequence data generated from different cDNA libraries, we discovered a large number of viral or viral related sequences, such as PMV and PstV. TSWV sequences were also present but at a lower frequency. The genome sequences of PMV (AF023848) and PStV (U05771) have long stretches of poly(A)s, which may serve as potential priming sites for first strand cDNA synthesis using oligo dT primer; while TSWV (AAC33560, AY870390, AY870391) genomic sequences do not exhibit long poly(A) tails. Therefore, we detected a large number of PMV and PStV sequences and a few numbers of TSWV.

PMV was first reported in the United States in 1965 (Kuhn, 1965) and its worldwide distribution is contributed by seed transmissibility. Analysis revealed that the majority of sequences in contig 0008 (586/605 or 96.8%) were derived from leaf tissues and the rest (19/605 or 3.2%) came from seed tissues. Tifrunner leaf tissues showed a similar percent of PMV sequences (277/6104 or 4.54%) compared to GT-C20 leaf tissues (309/8328 or 3.71%). This observation may partly be explained by the fact that PMV can be transmitted by aphids (*Aphis craccivora* and *Myzus persicae*) in a nonpersistent manner (Sreenivasulu and Demski, 1988). Analysis of single nucleotide polymorphisms (SNPs) among sequences in the same contig revealed an 89 nucleotide difference among 3850 bp or 2.31% sequence variation.

PstV, first detected in United States in 1982, was introduced into the United States through seeds from exotic germplasms (Demski and Lovell, 1985). Analysis of sequences in contig 0014 revealed that a majority (326 out of 330 or 98.8%) were derived from leaf tissues, with the remaining 4 sequences (1.2%) from seeds. Similar to PMV, PStV can be transmitted by aphids in a nonpersistent manner (Sreenivasulu and Demski, 1988), which could explain why PStV sequences were observed predominantly in leaf tissues versus seeds. Both PMV and PStV are potyviruses which represent a major genus in the *Potyviridae* family. They are composed of a (+) single stranded RNA molecule about 10 kb in length and consist of many coding regions which are processed to produce several functional domains (Urcuqui-Inchima *et al*., 2001). The RNA genome contains a viral protein genome-linked (VPg) covalently bound to the 5' end and a poly(A^+) at the 3' end (Fig. 1). VPg may act as a primer for RNA replicase through interaction with viral RNA polymerase (Fellers *et al*., 1998). It may also be involved in cell-to-cell movement of the virus through plasmodesmata (Rajamaki and Valkonen, 2002).

RT-PCR analyses have confirmed the presence of PMV and PStV transcripts in leaves, seeds and roots of all samples for both Tifrunner and GT-C20, with leaf tissue showing more intense bands relative to seeds. This observation is consistent with the frequencies of PMV sequences observed versus seed tissues. The presence of peanut plants infected with either PMV and/or PStV has been reported in peanut by previous studies (Kuhn, 1965; Kuhn *et al*., 1984). Our findings showed that they are both present in the same samples analyzed. The extent disease susceptibility by PMV or PStV alone or in combination in this case can not be ascertain since all plant analyzed showed the presence of both PMV and PStV transcripts. Plants that have been infected by a mild viral strain can have cross-protection against a more pathogenic virus of a similar or different strain (Freitas and Rezende, 2008; Ravelonandro *et al*., 2008).

The success of viral infection, the effective movement throughout the plant and the susceptibility of the plant are all important factors in causing plant disease symptoms. Along with the presence of PMV and PStV, 4 transcripts coding for a potential peanut responsive protein were observed. The consensus sequence (contig 0778) matched to Potyvirus VPg-interacting protein (PVIP), a plant-specific protein. The function for PVIP is not known, however protein translation identified a PHD finger-like domain that may be involved in transcriptional control in plant viral defense (Schindler *et al*., 1993). Using RNA interference (RNAi)-based gene silencing of PVIP in *Arabidopsis*, Dunoyer *et al*. (2004) showed that the protein acts an ancillary factor that enhances potyvirus infection and movement. In peanut, a similar strategy could be used to determine the putative PVIP involvement in potyvirus resistance.

TSWV sequences were also observed to be included in peanut ESTs. TSWV, a *Tospovirus* in the family of *Bunyaviridae*, is one of the most widely distributed and economically important plant viruses (Culbreath *et al*., 1997). It is transmitted by several species of thrips and causes devastating disease symptoms in plants such as peanut, pepper, tobacco and tomato in the Southeast part of the United States (Mandal *et al*., 2006). In this study, the number of sequences that matched TSWV was very low, because the nature of TSWV genome sequence is without a poly(A) tail and the techniques used for cDNA library construction. There were no TSWV sequences identified in leaf libraries and only 6 sequences were observed in seed libraries. Since TSWV genome does not

have long stretches of poly(A)s at the 3' end, we did not expect that TSWV sequences would be efficiently primed by oligo dT. The number of TSWV sequences in peanut ESTs probably does not reflect the true level of viral RNAs in the peanut tissues.

RT-PCR using TSWV specific primers (TSWV 722 and 723) (Adkins and Rosskopf, 2002) confirmed the presence of TSWV transcripts in some samples collected (Fig. 2). In GT-C20 leaf samples, RT-PCR analysis resulted in a higher number of TSWV positive leaf samples. Root samples were also shown a higher number of TSWV positive than leaf samples. This is consisted with observation by Rowland *et al.* (2005) that below-ground tissues showed a greater incidence of infection with TSWV than did leaves from the same plants. The presence of TSWV sequences in seed EST is interesting since there are no published reports on TSWV seed transmission in peanuts. However, there has been a report on the presence of TSWV in different parts of the peanut pods (shell, testa and cotyledons) using ELISA and RT-PCR on TSWV symptomatic plants (Pappu *et al.*, 1999). To confirm the presence of TSWV in peanut seeds, we analyzed seed samples collected in 2008. No PCR products were produced utilizing TSWV specific primers indicating no presence of TSWV RNA (data not shown).

The success of (+) ssRNA virus to invade a host and replicate involves not just virus-encoded factors but also plant host factors (Thivierge *et al.*, 2005). Analysis of peanut ESTs resulted in the identification of several plant host proteins that may interact and regulate tobamovirus multiplication. *TOM1* and its homologs (*TOM3* and *THH1*) genes in *Arabidopsis thaliana* encode multi-pass transmembrane proteins that are necessary for the efficient multiplication of tobamovirus (Yamanaka *et al.*, 2000, 2002; Fujisaki *et al.*, 2006). Tobamovirus is known to infect many plant species and many have been isolated from Solanaceous plants. To date, there are no reports of any tobamovirus infection in peanut. The presence of putative Tobamovirus responsive protein, *Tobacco mosaic virus* (TMV) helicase domain-binding protein, provides further support to the potential presence of a Tombamovirus in peanuts. Up to date, only two cases were reported where TMV was reported infecting legumes (Golnaraghi *et al.*, 2004).

The PMV, PStV, TSWV and potentially tobamovirus (individually or in combination) can cause severe yield losses. Understanding viral-plant interactions can advance the understanding of this relationship and potentially provide methods that can control viral infection and spread throughout the plants. Furthermore, it may provide a means of enhancing the plant's defense system by molecular biotechnology resulting in enhanced plant health and increased yield.

ACKNOWLEDGMENTS

We thank Ernest Harris and Kippy Lewis for technical assistance in the field and the laboratory. We thank Steven Valles for critical review of this manuscript. This research was partially supported by funds provided by USDA Agricultural Research Service, USDA Multi-Crop Aflatoxin Elimination Project, Peanut Foundation and Georgia Agricultural Commodity Commission for Peanuts. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

Abbink, T.E.M., J.R. Peart, T.N.M. Mos, D.C. Baulcombe, J.F. Bol and H.J.M. Linthorst, 2002. Silencing of a gene encoding a protein component of the oxygen-evolving complex of photosystem II enhances virus replication in plants. Virology, 295: 307-319.

Adkins, S. and E.N. Rosskopf, 2002. Key west nightshade, a new experimental host for plant viruses. Plant Dis., 86: 1310-1314.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res., 25: 3389-3402.

Bandla, M.D., L.R. Campbell, D.E. Ullman and J.L. Sherwood, 1998. Interactions of tomato spotted wilt *Tospovirus* (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. Phytopathol., 88: 98-104.

Boote, K.J., 1982. Growth stages of peanut (*Arachis hypogaea* L.). Peanut Sci., 9: 35-40.

Bunting, A.H., R.W. Gibbons and J.C. Wynne, 1985. Groundnut (*Arachis hypogaea* L.). In: Grain Legume Crops, Summerfield, R.J. and E.H. Roberts (Eds.). Collins, London, ISBN: 0003830373, pp: 747-800.

Culbreath, A.K., J.W. Todd, D.W. Gorbet, F.M. Shokes and H.R. Pappu, 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis., 81: 1410-1415.

Demski, J.W. and G.R. Lovell, 1985. Peanut stripe virus and the distribution of peanut seed. Plant Dis., 69: 734-738.

Dunoyer, P., C. Thomas, S. Harrison, F. Revers and A. Maule, 2004. A cystein-rich plant protein potentiates *Potyvirus* movement through an interaction with the Virus genome-linked Protein VPg. J. Virol., 78: 2301-2309.

Fellers, J., J. Wan, Y. Hong, G.B. Collins and A.G. Hunt, 1998. *In vitro* interactions between a potyvirus-encoded, genome-linked protein and RNA-dependent RNA polymerase. J. Gen. Virol., 79: 2043-2049.

Flasinski, S. and B.G. Cassidy, 1998. Potyvirus aphid transmission requires helper component and homologous coat protein for maximal efficiency. Arch. Virol., 143: 2159-2172.

Freitas, D.M.S. and J.A.M. Rezende, 2008. Protection between strains of papaya ringspot virus-type w in zucchini squash involves competition for viral replication sites. Sci. Agric., 65: 183-189.

Fujisaki, K., G.B. Ravelo, S. Naito and M. Ishikawa, 2006. Involvement of *THH1*, an *Arabidopsis thaliana* homologue of the *TOM1* gene, is tobamovirus multiplication. J. Gen. Virol., 87: 2397-2401.

Gillaspie, A.G. Jr., R.N. Pittman, D.L. Pinnow and B.G. Cassidy, 2000. Sensitive method for testing peanut seed lots for *Peanut stripe* and *Peanut mottle viruses* by immunocapture-reverse transcription-polymerase chain reaction. Plant Dis., 84: 559-561.

Golnaraghi, A.R., N. Shahraeen, R. Pourrahim, S. Farzadfar and A. Ghasemi, 2004. Occurrence and relative incidence of viruses infecting soybeans in Iran. Plant Dis., 88: 1069-1074.

Gunasinghe, U.B., S. Flasinski, R.S. Nelson and B.G. Cassidy, 1994. Nucleotide sequence and genome organization of peanut stripe potyvirus. J. Gen. Virol., 75: 2519-2526.

Guo, B., X. Chen, P. Dang, B.T. Scully, X. Liang, C.C. Holbrook, J. Yu and A.K. Culbreath, 2008. Peanut gene expression profiling in developing seeds at different reproduction stages during *Aspergillus parasiticus* infection. BMC Dev. Biol., 8: 12-12.

Guo, B., X. Chen, Y. Hong, X. Liang, P. Dang, T. Brenneman, C. Holbrook and A. Culbreath, 2009. Analysis of gene expression profiles in leaf tissues of cultivated peanuts and development of EST-SSR markers and gene discovery. Int. J. Plant Genomics, 2009: 14-14.

Holbrook, C. and A. Culbreath, 2007. Registration of tifrunner peanut. J. Plant Registr., 1: 124-124.

Kormelink, R., M. Storms, J. Van Lent, D. Peters and R. Goldbach, 1994. Expression and subcellular location of the NSM protein of tomato spotted wilt virus (TSWV), a putative viral movement protein. Virology, 200: 56-65.

Kuhn, C.W., 1965. Symptomatology, host range and effect on yield of a seed-transmitted peanut virus. Phytopathology, 55: 880-884.

Kuhn, C.W., J.W. Demski, D.V.R. Reddy, C.P. Benner and M. Bijaisoradat, 1984. Identification and incidence of peanut viruses in Georgia. Peanut Sci., 11: 67-69.

Luo, M., P. Dang, B.Z. Guo, G. He, C.C. Holbrook, M.G. Bausher and R.D. Lee, 2005a. Generation of expressed sequence tags (ESTs) for gene discovery and marker development in cultivated peanut. Crop Sci., 45: 346-353.

Luo, M., P. Dang, M.G. Bausher, C.C. Holbrook, R.D. Lee, R.E. Lynch and B.Z. Guo, 2005b. Identification of transcripts involved in resistance response to leaf spot disease caused by *Cercosporidium personatum* in peanut (*Arachis hypogaea*). Phytopathology, 95: 381-387.

Luo, M., X. Liang, P. Dang, C.C. Holbrook, M.B. Bausher, R.D. Lee and B.Z. Guo, 2005c. Microarray-based screening of differentially expressed genes in peanut in response to *Aspergillus parasiticus* infection and drought stress. Plant Sci., 169: 695-703.

Lynch, R.E., J.W. Demski, W.D. Branch, C.C. Holbrook and L.W. Morgan, 1988. Influence of peanut stripe virus on growth yield and quality of florunner peanut. Peanut Sci., 15: 47-52.

Mandal, B., H.R. Pappu, A.S. Csinos and A.K. Culbreath, 2006. Response of peanut, pepper, tobacco and tomato cultivars to two biologically distinct isolate of *Tomato spotted wilt virus*. Plant Dis., 90: 1150-1155.

Pappu, S.S., H.R. Pappu, C.A. Chang, A.K. Culbreath and J.W. Todd, 1998. Differentiation of biologically distinct peanut stripe potyvirus strains by a nucleotide polymorphism-based assay. Plant Dis., 82: 1121-1125.

Pappu, S.S., H.R. Pappu, C.A. Chang, A.K. Culbreath and J.W. Todd, 1999. Localization of tomato spotted wilt virus (Genus *Tospovirus*, Family *Bunyaviridae*) in peanut pods. Peanut Sci., 26: 98-100.

Rajamaki, M.L. and J.P. Valkonen, 2002. Viral genome-linked protein (VPg) controls accumulation and phloem-loading of a potyvirus in inoculated potato leaves. Mol. Plant Microb. Interact., 15: 138-149.

Ravelonandro, M., P. Briard, M. Glasa and S. Adam, 2008. The ability of a mild isolate of a *Plum Pox Virus* to cross-protect against *Sharka* Virus. Acta Hort., 781: 281-286.

Richmond, K.E., K. Chenault, J.L. Sherwood and T.L. German, 1998. Characterization of the nucleic acid binding properties of tomato spotted wilt virus nucleocapsid protein. Virology, 248: 6-11.

Rowland, D., J. Dorner, R. Sorensen, J.P. Beasley and J. Todd, 2005. Tomato spotted wilt virus in peanut tissue types and physiological effects related to disease incidence and severity. Plant Pathol., 54: 431-440.

Schindler, U., H. Beckmann and A.R. Cashmore, 1993. HAT3.1, a novel *Arabidopsis* homeodomain protein containing a conserved cysteine-rich region. Plant J., 4: 137-150.

Sreenivasulu, P. and J.W. Demski, 1988. Transmission of peanut mottle and peanut stripe viruses by *Aphis craccivora* and *Myzus persicae*. Plant Dis., 72: 722-723.

Stromvik, M.V., F. Latour, A. Archambault and L.O. Vodkin, 2006. Identification and phylogenetic analysis of sequences of *Bean pod mottle virus, Soybean mosaic virus* and *Cowpea chlorotic mottle virus* in expressed sequence tag data from soybean. Canadian J. Plant Pathol., 28: 289-301.

Takeda, A., K. Sugiyama, H. Nagano, M. Mori, M. Kaido, K. Mise, S. Tsuda and T. Okuno, 2002. Identification of a novel RNA silencing suppressor, NSs protein of tomato spotted wilt virus. FEBS Lett., 532: 75-79.

Thivierge, K., V. Nicaise, P.J. Dufresne, S. Cotton, J.F. Laliberté, O. Le Gall and M.G. Fortin, 2005. Plant virus RNAs: Coordinated recruitment of conserved host functions by (+) ssRNA viruses during early infection events. Plant Physiol., 138: 1822-1827.

Thompson, J.R., K.L. Perry and W. De Jong, 2004. A new potato virus in a new linage of picorna-like virus. Arch. Virol., 149: 2141-2154.

Tsompana, M., J. Abad, M. Purugganan and J.W. Moyer, 2005. The molecular population genetics of the *Tomato spotted wilt virus* (TSWV) genome. Mol. Ecol., 14: 53-66.

Urcuqui-Inchima, S., A-L. Haenni and F. Bernardi, 2001. Potyvirus proteins: A wealth of functions. Virus Res., 74: 157-175.

Valles, S.M., C.A. Strong, P.M. Dang, W.B. Hunter and R.M. Pereira *et al*., 2004. A picorna-like virus from the red imported fire ant, *Solenopis invicta*: Initial discovery, genome sequence and characterization. Virology, 328: 151-157.

Valles, S.M., C.A. Strong, W.B. Hunter, P.M. Dang, R.M. Pereira, D.H. Oi and D.F. Williams, 2008. Expressed sequence tags from the red imported fire ant, *Solenopsis invicta*: Annotation and utilization for discovery of viruses. J. Invertebr. Pathol., 99: 74-81.

Yamanaka, T., T. Ohta, M. Takahashi, T. Meshi and R. Schmidt *et al*., 2000. *TOM1*, an Arabidopsis gene required for efficient multiplication of a tobamovirus, encodes a putative transmembrane protein. Proc. Natl. Acad. Sci., 97: 10107-10112.

Yamanaka, T., T. Imai, R. Satoh, A. Kawashima and M. Takahashi *et al*., 2002. Complete inhibition of tobamovirus multiplication by simultaneous mutations in two homologous host genes. J. Virol., 76: 2491-2497.